FINAL REPORT

INVESTIGATION OF METHODS FOR THE STERILIZATION OF POTTING COMPOUNDS AND MATED SURFACES

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BECTON, DICKINSON RESEARCH CENTER
BECTON, DICKINSON AND COMPANY
RALEIGH, NORTH CAROLINA

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Investigation of Methods for the Sterilization of Potting Compounds and Mated Surfaces

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Submitted by:

J. J. Tulis

J. J. Tulis, Ph. D.
Director, Microbiological Sciences Department
Becton, Dickinson Research Center

Approved by:

G. Briggs Phillips

G. Briggs Phillips, Ph. D.
Director
Becton, Dickinson Research Center

Becton, Dickinson and Company
Raleigh, North Carolina

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ABSTRACT

Studies have been conducted on the feasibility of sterilizing potting compound at temperatures ranging from ambient to 125C by addition of sterilant chemicals (formaldehyde-liberating synthetic resins and polymers) to the potting compound during the curing process. Evidence was obtained which indicated that the polymeric form of monomeric formaldehyde, paraformaldehyde, was considerably more effective as a sterilizing agent than were the formaldehyde-resins used. With a concentration of 1 mg paraformaldehyde in 100 cc air, routine sporicidal activity (inactivation of 1×10^5 bacterial spores) was achieved within 72 hours at 22C, 14 hours at 35C, 5 hours at 60C, and 1 hour at 125C. Analytical calculations showed that a maximum of 1×10^5 molecules of monomeric formaldehyde per bacterial spore were required for inactivation. Potting compound-sterilant mixtures proved inhibitory against both Gram positive and negative microorganisms, the degree of inhibition being a function of the test organism, sterilant, and sterilant concentration. Data from studies on the loss of monomeric formaldehyde from synthetic resins, paraformaldehyde, and potting compound-sterilant mixtures suggested that internal sublimation of sterilants with outward diffusion of

vaporized formaldehyde transpired. During this process of evolution of sterilizing gas, it was theorized that embedded bacterial spores were subjected to in situ inactivation with the resultant sterilization of the entire component. Experimental verification for internal sterilization of potting compound was subsequently obtained by employing a spore-embedding technique described in the report. Inactivation of 1×10^5 embedded bacterial spores was achieved at temperatures ranging from 45 to 90C within relatively short time periods with the use of melamine formaldehyde or paraformaldehyde as the sterilant additive. Preliminary studies using DMSO in conjunction with monomeric formaldehyde suggested that sporicidal activity was enhanced using this combination.

The concept of a "self-sterilizing" potting compound has been demonstrated. Procedures were developed that should ensure the internal sterility of electronic spacecraft components. The applicability of this development to the NASA spacecraft sterilization program could result in a reduction of the total encapsulated microbial burden on spacecraft, thereby allowing a shorter and less thermally destructive heat sterilization cycle. Also this technique might be applicable to the preparation of sterile science instruments being designed for use in the Viking program and other subsequent planetary explorations.

INTRODUCTION

This report presents the research conducted by Becton, Dickinson and Company during the period of August 13, 1968 through October 13, 1969 in support of the planetary quarantine requirements of the National Aeronautics and Space Administration under Contract NASw-1764. Research management was provided by Dr. G. Briggs Phillips, Director, Becton, Dickinson Research Center (BDRC), and Dr. J. J. Tulis, Director, Microbiological Sciences, BDRC, acting as Project Officer and Assistant Project Officer, respectively. The consultative services of Dr. J. H. Brewer are gratefully acknowledged.

The subject research program was instituted as the result of a proposal prepared by Drs. Brewer and Phillips, dated August, 1967, and submitted to the Office of Space Science and Applications, National Aeronautics and Space Administration. A one-year contract was entered into as of August 13, 1968 between NASA Headquarters and Becton, Dickinson and Company. A 2-month, no-cost extension of the contract to allow conclusion of laboratory research and finalization of this report was approved, thereby extending the contract through October 12, 1969.

Technical briefings on the status of the contract research were presented at the NASA Spacecraft Sterilization Technology Seminars held at Cape Kennedy on February 12, 1969 and the Southwestern Radiological Health Laboratory, Las Vegas on September 24, 1969.

Data presented in this report are concerned with the successful development of methodology for the internal sterilization of potting compound. It was recognized early in the contract studies, after preliminary experimentation had been completed, that maximal effort should be expended towards the satisfactory completion of the indicated task, namely, the internal sterilization of potting compound, because of the formidable challenge posed by this problem. In order to achieve our goal, however, it was considered important that the temperature parameters as originally stated be expanded. Therefore, the experimental temperature range was altered and studies were conducted from ambient to 125C rather than 45 to 60C. The rationale for this temperature range was 2-fold; namely, studies conducted at 125C would be applicable to the vast knowledge that NASA has accumulated on dry heat sterilization at this temperature, and the known interest in sterilization of heat-sensitive materials at temperatures

slightly above ambient. In addition, the polymeric form of formaldehyde, paraformaldehyde, was included in the contract studies along with the formaldehyde containing organic resins. Thus, previously acquired knowledge gained at Becton, Dickinson and Company on paraformaldehyde could be applied to the contract research. Also, at the suggestion of NASA, preliminary studies on the combined effect of formaldehyde gas and dimethyl sulfoxide (DMSO) were conducted. During this contract period, only limited studies on mated surfaces were performed. It was felt that our approach would most directly reach a scientific milestone which would be profitable to NASA; and, in the final analysis, much of the knowledge gained in the studies on the sterilization of potting compound could be applied to the sterilization of mated surfaces.

The potting compound used in our studies was selected as a convenient representative electronic material that would best facilitate the exploratory studies. It was necessary that the material used not be highly exothermic and lend itself to the laboratory procedures required to conduct sterilization feasibility studies. Dow Corning RTV-3140 satisfied these requirements and

was used as a representative potting compound throughout our studies. Testing of a broad range of potting compounds was obviously beyond the scope of this preliminary investigation.

BACKGROUND

In recent years there have been a number of attempts to produce coatings or product additives that will result in materials with "self-sterilizing" properties (9,12). Generally, little or no success in achieving sterility has resulted from the use of non-volatile or low-vapor-pressure chemicals (9). This is primarily because such chemicals usually require high moisture environments for maximum bactericidal activity (6,8,10) and, even in this instance, show only limited sporicidal activity. On the other hand, some success has been experienced in the development of self-sterilizing materials that incorporate volatile-type disinfectants (9). Nevertheless, the usefulness of a volatile germicide is maximized if volatility is very low at ambient temperatures yet significant at elevated temperatures below 100C.

Studies conducted at Becton, Dickinson and Company laboratories prior to initiation of the contract studies demonstrated the potential usefulness to spacecraft sterilization problems of organic chemicals containing a volatile chemical germicide. Specifically, it appeared feasible and practical that sterilant mixtures could be developed that would be relatively inactive at

ambient conditions but when placed at moderately elevated temperatures, would evolve sufficient amounts of sterilant to inactivate large numbers of bacterial spores.

The rationale for our studies was based on the knowledge that various organic resins and polymers, when exposed to elevated temperature, will release potentially sterilizing quantities of gaseous formaldehyde along with the required amount of moisture. The active formaldehyde evolves from the organic resin or polymer in such a manner that the rate of release, and therefore the sterilization process, is a function of time and temperature. The amount of sterilant gas and moisture released is extremely small and contaminating microorganisms are theoretically subjected to in situ sterilization without untoward effects on the electronic parts, mated surfaces, or surrounding areas.

The chemical compounds under investigation possessed the common property of liberating formaldehyde gas upon elevation of temperature. Included in the studies were representative compounds of the synthetic resins and the polymer, paraformaldehyde. The synthetic resins used are reaction products of melamine, urea and phenol with formaldehyde. The major chemical properties of paraformal-

dehyde are listed in Table I, Appendix.

Melamine (2, 4, 6 triamine - 1, 3, 5 triazine) reacts under alkaline conditions with formaldehyde to give various methylol derivatives (17). Because six reactive sites are available, the hexamethylol melamine compound can be prepared. However, the most commonly used product is the methylated trimethylol melamine (17), the structure of which is depicted in Figure 1, Appendix. Cationic resins can be formed with melamine formaldehyde under acidic conditions (16), which are used to impart wet strength properties to paper and shrink resistance properties to textiles.

The reaction products of urea and formaldehyde are a mixture of mono-methylol urea and di-methylol urea (16). The mono-methylol urea compound, with only one formaldehyde reactive group, is not as effective in imparting stability to cellulosic fabrics as is the di-methylol urea (17), the structure of which is shown in Figure 2, Appendix. Urea formaldehyde is used in the paper and textile industry to provide desired properties to these materials, either as a precondensate (i.e., mixture of mono- and di-methylol urea) which penetrates the cellulose fibers to form the 3-dimensional resin in situ or as a partial condensate which

does not penetrate the cellulose fibers but instead forms the 3 dimensional resin on the fiber surface (16). Aqueous urea formaldehyde solutions differ from formalin solutions by containing methylol ureas of low molecular weight and water-soluble urea formaldehyde condensates; some free and loosely bound hydrated formaldehyde (methylene glycol) is present (28). Aqueous formaldehyde (formalin) is composed mainly of methylene glycol and various polymeric hydrates (polyoxymethylenes); very little unhydrated monomeric formaldehyde is found (21).

The phenol formaldehyde resins represent many complex products which are produced by reacting formaldehyde with various phenolic compounds (25). The reaction of formaldehyde with the phenols can be catalyzed by both acidic and alkaline conditions thereby resulting in products ranging from simple methylol and methylene derivatives (Figure 3, Appendix) to very complex resins (26). Phenol formaldehyde resins are defined as "mixtures of polymethylene compounds in which phenolic radicals are linked by methylene groups" (27).

Paraformaldehyde is defined as "a mixture of polyoxymethylene glycols containing from about 90 to 99 per cent formaldehyde and a balance consisting primarily of free and combined water" (22). It is not a new compound, having

been first prepared in 1859 (4), and named "paraformaldehyde" in 1888 (19). The chemical composition of paraformaldehyde is shown in Figure 4, Appendix, where "n" may represent from 8 to 100 formaldehyde units. Because of the relative insolubility of paraformaldehyde in acetone, a property not exhibited by low molecular weight polyoxymethylene glycols, the majority of the polymeric forms of paraformaldehyde contain more than 12 formaldehyde units (23). In appearance, paraformaldehyde is a colorless solid, which may be flaky, granular, or a fine powder. At ambient temperatures, paraformaldehyde slowly vaporizes releasing monomeric formaldehyde gas; probably accompanied by water vapor (14). The rate of depolymerization is a function of heat and availability of polymeric end groups (24), since depolymerization occurs at the hydroxyl end groups as an "unzippering" reaction. Thus, paraformaldehyde composed of high molecular weight polymers will evolve gas more slowly than that composed of low molecular weight polymers, although the depolymerization rate is the same for both. The end result of polymer breakdown is the formation of water from the terminal methylene glycol residue. With improvements in processing, the paraformaldehyde manufactured today has a relatively narrow molecular weight range.

Paraformaldehyde has been used as a fungicide and bactericide for some years. Kaitz (11), in 1956, described the use of the fine white powdered paraformaldehyde for the production of formaldehyde gas, a process subsequently used extensively in the poultry industry (20). The use of paraformaldehyde as a surface sterilant and detoxifying agent was described in a recent publication by Taylor et al (18).

The decision to include paraformaldehyde as a candidate for incorporation into the potting compound in order to achieve sterility was founded on the knowledge that this material is an excellent source of monomeric formaldehyde gas which can be produced in a temperature-controlled reaction. Because paraformaldehyde depolymerizes completely to yield pure monomeric formaldehyde there are no contaminating residues as found with formalin solutions (methanol and formic acid residues) or the various synthetic resins (complex resin residues). Thus, paraformaldehyde provided us with the best available source of formaldehyde gas for assessment of the potential applicability of the synthetic resins in spacecraft component sterilization.

RESEARCH OBJECTIVES

The overall purpose of this research was to investigate and develop a useful method for sterilizing potting compound at temperatures above ambient but below those usually required for dry heat sterilization. The specific objectives of the research were to evaluate the feasibility of using various resins or polymers for sterilizing potting compound at various temperatures, to determine on the basis of appropriate studies how the sterilant mixtures could be most effectively applied, to select candidate systems for more extensive sterilization kinetics studies, and to develop methodology for the verification of internal sterility of potting compound.

By adhering to a systematic research approach, answers have been provided for the following relevant questions.

1. What are the comparative sporicidal properties of the synthetic resins and paraformaldehyde?

2. Will sufficient sterilizing gas be released from /ug amounts of potential sterilant chemicals to inactivate 1 x 10^5 bacterial spores within a meaningful time period?

3. What is the relationship of temperature to the efficacy of the sterilization process when using the formaldehyde-liberating chemicals?

4. Can the various sterilant additives be mixed with potting compound without altering the gross or obvious characteristics of the material?

5. Are the mixtures of RTV and sterilant sufficiently biocidal to inhibit microbial growth?

6. What are the comparative biocidal properties of the various RTV-sterilant mixtures as revealed by the zone inhibition test? What is the effect of sterilant concentration?

7. What are the relative sensitivities of various Gram positive and negative bacteria to the RTV-sterilant mixtures?

8. What are the kinetics of gaseous evolution from melamine formaldehyde and paraformaldehyde at temperatures ranging from ambient to 125C?

9. How do the kinetics of gaseous evolution from resin-RTV and polymer-RTV compare to those of the pure chemicals?

10. What is the molecular relationship of formaldehyde gas inactivation of bacterial spores when using various donors of formaldehyde gas? When the gas is released at different rates?

11. What happens to the biocidal properties of RTV-sterilant mixture upon exposure to elevated temperatures?

12. Can sterilization of bacterial spores be achieved after prior exposure of RTV-sterilant mixtures to elevated temperatures?

13. How can information on the temperature controlled release of formaldehyde from melamine formaldehyde and paraformaldehyde be useful to NASA?

14. Can microorganisms entrapped in potting compound be inactivated by the internal evolution of formaldehyde gas? At what rate? At what temperature? With what additive and concentration?

15. What is the sterilizing effect of DMSO when used in combination with formaldehyde gas?

16. What milestones have been reached by these studies? How can these studies be useful to NASA?

17. What course of research is being pursued in future NASA-supported contract studies?

EXPERIMENTAL PROCEDURES

Studies on the inactivation of bacterial spores were conducted with the use of B-D certified biologic indicators. These included spore strips inoculated with either Bacillus stearothermophilus (1×10^5) or Bacillus subtilis var niger (B. globigii, 1×10^5).

The test procedure used for evaluation of the biocidal properties of the various formaldehyde-liberating organic chemicals involved the placement of mg amounts of sterilant additive into 100 cc glass vessels, the stoppers of which were affixed with metal hooks onto which the filter paper spore strips were attached (Figure 5, Appendix). Stoppered vessels were sealed with paraffin prior to placement in incubators or ovens at temperatures ranging from ambient to 125C for evaluation of the sterilizing efficiency of the various additives as a function of time and additive concentration. Trials were conducted in triplicate and each parametric point was repeated a minimum of 2 times; the majority were repeated more often. Spore strips were assayed for viability by inoculation into trypticase soy broth (TSB) with appropriate incubation temperature (60C for

the thermophilic sporeformer B. stearothermophilus and 37C for the mesophilic sporeformer B. globigii) for periods of at least 4 days with daily observation for presence of growth. Positive cultures were examined for possible contaminating microorganisms in order to eliminate false positives.

Studies on the addition of organic sterilants to potting compound were conducted with the use of specific amounts of organic resin or polymer and Dow Corning RTV 3140. The sterilant-potting compound combinations were mixed thoroughly and then allowed to cure for 48 hours at ambient temperature, during which time a negligible weight loss occurred (Table II, Appendix). To each 25 ml of uncured RTV the following amounts of melamine formaldehyde or paraformaldehyde were added: 0.475 and 2.375 g melamine formaldehyde to give 1.9 and 9.5% additive, respectively; and 0.250 and 1.250 g paraformaldehyde to give 1% and 5% additive, respectively. The total weight of resin in potting compound was 5.9 mg and 29.4 mg for the 1.9% and 9.5% discs, respectively; and the total weight of polymer in potting compound was 3.1 mg and 15.5 mg for the 1% and 5% discs, respectively. By using the above concentrations of additive, the available formaldehyde residue in paraformaldehyde and melamine formaldehyde were comparable.

For zone inhibition tests using a modification of the antibiotic disc sensitivity procedure, 10 mm RTV-sterilant discs were prepared and placed onto trypticase soy agar surfaces previously inoculated with 24-hour cultures of the various test organisms. Included in these studies were Bacillus globigii, Serratia marcescens, Klebsiella pneumoniae, Staphylococcus aureus, and Escherichia coli. Zone inhibition readings were made at 24, 48 and 72 hours after inoculation; zone diameters were measured in mm's.

The methodology employed in the determination of formaldehyde gas loss from melamine formaldehyde and paraformaldehyde as a function of time and temperature included series of accurate weight assays using an analytical digital Mettler balance. Appropriate control samples were routinely included in all trials. Individual experimental points were assayed in triplicate and repeated at least twice; 100 mg samples of resin or polymer were used in aluminum weighing pans. The studies on loss of formaldehyde gas from the resin-RTV and polymer-RTV mixtures were conducted in a similar manner; results were corrected for any weight loss recorded for control discs.

Preliminary studies on the recovery of bacterial spores from RTV, using a glycol ether for melting of the RTV, indicated that this solvent was too deleterious to the spores to be useful. An alternate method was developed which proved quite satisfactory in the recovery of spores from RTV, although not selected for detailed studies because of low recovery efficiency. The procedure involved the sectioning of RTV matrices, using a plexiglass template and stainless steel surgical blade. The resulting numerous small equal size cubes were placed in buffer and the surface spores removed by insonation. The comparative recovery of viable spores from internal surfaces of treated and untreated RTV would theoretically provide information on the internal sterilization capabilities of the additives as a function of time and temperature. As indicated, because of the low recovery of viable spores, further studies using this method were abandoned. Additional exploratory research involved the use of contaminated sutures that were sealed within RTV, exposed to certain experimental conditions, and removed for assay. Problems were encountered in the removal of sutures from the cured RTV, and this procedure was also dropped.

Thus, after employing a number of laboratory procedures for verification

of internal sterility, the following method was selected as the most reproducible, realistic, and efficient. Biologic indicators (filter paper strips inoculated with 1×10^5 bacterial spores) were placed within 0.5 mil plastic (Teflon) pouches, sealed, and then embedded within RTV, with or without additive, prior to the curing process (Figure 6, Appendix). The actual method involved the use of 25 ml uncured RTV-sterilant mixtures, one-half of which was placed into Petri plates. From 3 to 9 sealed biologic indicators were then placed onto the surface of the uncured RTV-sterilant mixture and the remainder of the 25 ml volume poured over the plastic pouches containing the bacterial spores. The plates were allowed to cure at ambient temperature for 72 hours prior to further evaluation at elevated temperatures. Control studies indicated that sufficient formaldehyde gas was not generated during the curing process to completely inactivate the biologic indicators. Additional studies where embedded spores were held for 96 hours at ambient temperature gave the same results. The embedded spores were subjected to various time/temperature periods, after which the strips were removed aseptically and assayed qualitatively for viable spores by inoculation into TSB. Studies were conducted in

triplicate at 45, 60, and 90C.

Studies on the effect of DMSO when used in conjunction with formaldehyde gas were conducted by saturating biologic indicators with undiluted DMSO and subsequently placing on hangers in 100 cc vessels as previously described. Formaldehyde gas was produced from paraformaldehyde by temperature elevation. Strips were removed after specified time periods and assayed for viable spores.

RESULTS

Biocidal Properties of Synthetic Formaldehyde-Containing Resins and the Polymer, Paraformaldehyde.

Empirical evidence has been obtained which indicated that the polymeric form of formaldehyde, paraformaldehyde, was considerably more effective as a sterilizing agent than were the formaldehyde-resins. As indicated in Table I, although 1×10^5 bacterial spores could be killed by exposure to melamine formaldehyde (at 6 mg/100 cc air) for 24 hours at 45 or 60C, it was not effective at lesser concentrations. Inactivation was not achieved at any of the concentrations used at 22 or 35C within 24 hours and inconsistencies in killing were observed in replicate tests.

The biocidal properties exhibited by urea formaldehyde, when used as an aqueous solution are shown in Tables II and III. Due to the viscous nature of the resin and general insolubility in organic solvents, aqueous solutions of urea formaldehyde were prepared for evaluation of sporicidal activity. Preliminary studies showed that 1 to 3 ml of a 50% urea formaldehyde solution were sporicidal within 24 hours at 22, 35, or 60C. Information presented in Table II,

where 2.5 and 5.0 mg urea formaldehyde were used at 60C in a test method identical to that used for melamine formaldehyde, shows complete inactivation of mesophilic spores of B. globigii within 2 to 3 hours whereas similar inactivation of thermophilic spores of B. stearothermophilus required 4 hours at the higher concentration of additive. Preliminary findings on the feasibility of using aqueous urea formaldehyde, which is a mixture of low molecular weight methylol ureas and urea formaldehyde condensates, as a sterilizing spray was demonstrated by the inactivation of 1×10^5 bacterial spores on aluminum strips following the spray application of a 1% urea formaldehyde solution (Table III). Sprayed strips were air-dried and then placed at 45 or 60C for 1, 2, or 3 hours; complete kill of bacterial spores was achieved in 1 hour at both 45 and 60C.

By comparison, when using paraformaldehyde under similar test conditions but at much lower concentrations (1 mg/100 cc air), routine sporicidal activity was achieved within 72 hours at 22C, 14 hours at 35C, 5 hours at 60C, and 1 hour at 125C (Table IV).

Information on the biocidal activity of paraformaldehyde (2 mg/100 cc air) at 35C is shown in Table V as a function of the time required for visible

microbial growth to become evident after placement of exposed biologic indicators in sterility test media. When biologic indicators were exposed to formaldehyde gas from paraformaldehyde for a period of 4-7 hours, no difference in microbial growth as compared to controls was observed. However, a 9-10 hour and a 12 hour exposure were associated with a delay in microbial growth attributable to a partial inactivation of the initial spore inoculum. A 14-24 hour exposure was associated with routine inactivation of all bacterial spores.

The comparative inactivation of 2 sporeforming bacteria, B. stearothermophilis and B. globigii, by melamine formaldehyde or paraformaldehyde (2 mg/100 cc air) with 30 minutes to 3 hours exposure at 125C is shown in Table VI. The spores of both Bacillus species were inactivated within about 1 hour by paraformaldehyde, whereas at least 3 hours were required for comparable inactivation by melamine formaldehyde. The differential in bacterial species sensitivity observed in previous studies with urea formaldehyde (Table II) wherein mesophilic spores were more sensitive than thermophilic spores to apparent gaseous inactivation was not evident in these results. It is important to recognize, however, that bacterial inactivation per se at 125C was a compounded effect of heat and

sterilizing gas for both bacterial species whereas at 60C only the mesophiles were exposed to this combination of lethal effects.

Gaseous Evolution of Monomeric Formaldehyde from Melamine Formaldehyde, Paraformaldehyde, and Potting Compound-Sterilant Mixtures at Elevated Temperatures with Concurrent Loss of Biocidal Activity

In studies on the kinetics of evolution of formaldehyde gas, it was observed that at 60C approximately 10% loss of formaldehyde occurred within 6 hours from the pure chemical paraformaldehyde and from 5% paraformaldehyde-RTV discs, respectively. The biocidal activity of the RTV-sterilant discs was not reduced appreciably during this time as revealed by the zone inhibition test procedure. The loss of formaldehyde from paraformaldehyde continued progressively upon prolonged exposure at 60C with a 73% loss observed after 168 hours.

The loss of gaseous formaldehyde from melamine formaldehyde at 60C after 6 hours was approximately 3% and 2% from the resin and from 9.5% resin-RTV discs, respectively. Some loss of biocidal activity was observed after 4-hours exposure of the resin-RTV discs to 60C. The loss of available formaldehyde from the resin continued until 72 hours, when a 10% loss was recorded and did not increase significantly throughout the 168-hour exposure period. The comparative

loss of formaldehyde from paraformaldehyde and melamine formaldehyde over a 168-hour period is depicted in Figure 1.

As shown in Figure 2, at 90C exposure approximately 52% loss of formaldehyde took place within 6 hours from paraformaldehyde. In comparison, about 9% loss of formaldehyde occurred within the same time period from the resin, melamine formaldehyde. Within 24 hours the respective loss of formaldehyde from the polymer and resin were 82% and 10%. At 168 hours, when all available formaldehyde had evolved from paraformaldehyde, only 14% loss was observed for melamine formaldehyde.

At 125C exposure, the comparative loss of formaldehyde as a gas from the polymer paraformaldehyde and the resin melamine formaldehyde was 95% and 6% after 1 hour, 98% and 9% after 2 hours, 98% and 10.5% after 6 hours, and 100% and 24% after 7 days, respectively (Figure 3). The findings indicated that paraformaldehyde completely depolymerized and vaporized at 125C within several hours, whereas after 7 days exposure, melamine formaldehyde had lost approximately one-fourth of available formaldehyde residue.

Illustrated in Figure 4 are the comparative loss of gaseous formaldehyde from paraformaldehyde-RTV and melamine formaldehyde-RTV discs at 90C and the resultant progressive decrease in biocidal activity which transpired. The average inhibition zones after 1, 2, 4, and 6 hours prior exposure of paraformaldehyde-RTV to 90C were 23, 22, 16 and 12 mm in diameter; no biocidal activity was evident after 24 hours. The zones of inhibition produced by melamine formaldehyde-RTV discs, although not as large initially as those produced by the paraformaldehyde-RTV discs, were indicative of a slower rate of loss of biocidal activity throughout the 24-hour exposure period.

Studies on the quantitative release of gas from the polymeric form of formaldehyde, paraformaldehyde, are shown in Figure 5. Loss of formaldehyde through vaporization was recorded for a 168-hour period at both ambient (23C) and 45C; findings were extrapolated to 100% loss. Depicted are the weight losses recorded and the extrapolated linear expressions which resulted in a 100% loss at 45C within 12.5 days and at ambient temperature within 47 days, respectively.

A summary presentation of data on the loss of formaldehyde from paraformaldehyde and melamine formaldehyde at temperatures from 45C to 125C and exposure

periods of 1 hour through 7 days is shown in Table VIII. Significant differences in gaseous evolution from the two formaldehyde-liberating chemicals as a function of temperature can be seen.

Microbial Inhibition of Potting Compound-Sterilant Mixtures

Results from studies on the inhibition of bacteria by RTV potting compound discs containing various concentrations of organic resin or polymer are presented in Table VII. Discs containing 1% paraformaldehyde displayed good inhibition against *Bacillus globigii*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli* with zones of 15 to 32 mm diameter. At 5% paraformaldehyde, inhibition zones of 26 to 44 mm diameter were observed. Similarly prepared discs containing melamine formaldehyde or urea formaldehyde showed inhibition only against *S. aureus* at 1% resin additive with 16 mm and 15 mm diameter zones, respectively. At 5% resin additive, the melamine formaldehyde discs were somewhat more inhibitory than the urea formaldehyde discs, displaying inhibitory properties against all test organisms except *K. pneumoniae*. Five per cent urea formaldehyde discs showed no inhibition against *S. marcescens* and *E. coli*. At 10% resin additive, discs prepared with either resin showed

inhibition zones of 15 to 24 mm diameter. Representative zones of inhibition are shown in Figure 6.

In related studies, when discs containing 1% paraformaldehyde were placed within 100 cc sealed vessels, sufficient sterilizing gas was released within 4 to 6 hours at 90C to kill 1×10^5 bacterial spores. Under similar conditions, 5% resin impregnated discs were not effective. Therefore, studies were conducted on the exposure to elevated temperatures of potting compound discs containing sterilant additive prior to biologic assessment for residual biocidal activity. Results indicated that a 1-hour exposure at 125C or 5-hour exposure at 60C allowed sufficient residual formaldehyde to remain within discs orginally containing 1% paraformaldehyde to inactivate 1×10^5 bacterial spores upon subsequent placement in 100 cc sealed vessels for 1 hour at 125C.

Inactivation of Embedded Bacterial Spores by Internal Evolution of Monomeric Formaldehyde

The data presented in the following series of tables shows the results of studies on the inactivation of RTV-embedded bacterial spores as a consequence of internal formaldehyde gas evolution from melamine formaldehyde or paraformaldehyde. Studies were conducted at 45C, 60C, and 90C; inactivation of bacterial

spores was examined as a function of time, additive, and additive concentration at the indicated experimental temperatures.

Using 9.5% melamine formaldehyde-RTV or 5% paraformaldehyde-RTV at 45C (Table IX) complete kill of embedded spores occurred after 24-hours exposure to paraformaldehyde, whereas incomplete inactivation was observed after 96-hours exposure to melamine formaldehyde. With a lesser concentration of available formaldehyde, i.e., 1.9% melamine formaldehyde or 1% paraformaldehyde (Table X), a similar inactivation profile was observed for paraformaldehyde; melamine formaldehyde proved ineffective in producing sterility after 96-hours exposure.

At 60C exposure, using the higher concentrations of resin or polymer additive (Table XI), complete inactivation of internally placed spores was achieved after 2-hours exposure to paraformaldehyde-RTV and 36-hours exposure to melamine formaldehyde-RTV. As observed, partial inactivation (i.e., some replicate spore strips completely sterile) took place after 1 hour and 12-hours exposure to paraformaldehyde and melamine formaldehyde, respectively. In comparison, when using the lesser concentrations of sterilant additive (Table XII), 6 hours of exposure to paraformaldehyde were required for complete kill and incomplete kill was observed after 96-hours exposure to melamine formaldehyde.

Information is presented in Table XIII on the inactivation of embedded bacterial spores at 90C when using 5% paraformaldehyde or 9.5% melamine formaldehyde. Complete sporicidal activity was achieved after only 15-minutes exposure to the formaldehyde polymers and a 1-hour exposure to the formaldehyde resin. Of interest was the partial inactivation of embedded bacterial spores after 18-hours exposure at 90C to RTV alone, a finding that has been reported by others (1). When using the lesser concentrations of sterilant additives (Table XIV), the time required for complete inactivation of bacterial spores was slightly extended, i.e., 30 minutes and 2 hours for the paraformaldehyde and melamine formaldehyde, respectively.

Summary data on the inactivation of RTV embedded bacterial spores as a function of additive, additive concentration, time and temperature is shown in Table XV. Appreciable differences between the time required for apparent sterilization of RTV potting compound can be seen when comparing additive and temperature, whereas the 5-fold difference in additive concentration did not effect the kill rate significantly.

Sporicidal Activity of DMSO and Monomeric Formaldehyde

Preliminary studies on the sporicidal activity of paraformaldehyde when used in combination with dimethyl sulfoxide (DMSO) are shown in Table XVI. Spore strips were exposed to DMSO and subsequently exposed to formaldehyde gas liberated from paraformaldehyde. Although data indicated that both DMSO and paraformaldehyde were sporicidal to varying degrees when used alone, the inactivation of bacterial spores appeared enhanced when these agents were used in combination. At 60C, 1×10^5 bacterial spores were killed within 3 hours when subjected to a mixture of DMSO and paraformaldehyde, whereas with paraformaldehyde alone 5 hours were required for similar inactivation. At 90C, some inactivation occurred after 30 minutes exposure to both DMSO and paraformaldehyde and complete killing took place within 60 minutes. In comparison, when DMSO or paraformaldehyde were used alone, 120 minutes were required for similar inactivation.

DISCUSSION

Studies conducted at Becton, Dickinson and Company on the potential application of formaldehyde-liberating compounds in the successful sterilization of potting compounds have indicated that the overall concept is entirely feasible. Research was conducted on the inactivation of bacterial spores by the synthetic resins, melamine formaldehyde, urea formaldehyde, and phenol formaldehyde, and the organic polymer, paraformaldehyde, both as pure chemicals and as additives to RTV potting compound. Subsequent research was directed towards the procurement of data on the comparative loss of formaldehyde and biocidal activity from potting compound-sterilant mixtures as a function of various parameters, and development of methodology for the verification of the internal sterility of potting compound. Candidate sterilants selected for these latter studies included the synthetic resin, melamine formaldehyde, and the organic polymer, paraformaldehyde.

Without exception, the use of paraformaldehyde as a potential sterilant, in comparison with the synthetic resins, proved to be significantly more

effective. These results were not unexpected since we were aware that paraformaldehyde was potentially a better source of monomeric formaldehyde than were the synthetic resins. It was apparent from the studies, also, that the inactivation process per se was strictly a function of available formaldehyde gas. Analysis of our data on the biocidal activity of controlled quantities of paraformaldehyde at ambient temperatures and 60C (conducted in sealed 100 cc vessels) in conjunction with our data on the evolution of formaldehyde gas from paraformaldehyde as a function of time and temperature provided information on the maximal molecular amounts of formaldehyde gas required to inactivate microbial spores. Based on Avogadro's number, 1 mg formaldehyde contains 2×10^{19} molecules. Experimental evidence indicated that 1×10^{5} bacterial spores suspended in a 100 cc sealed vial and exposed to 1 mg paraformaldehyde would be inactivated within 5 hours. During this period of time, as studies on evolution of gas as a function of time and temperature revealed, approximately 7.5% of the available paraformaldehyde vaporized, i.e., about 75 µg. Calculations showed that 75 µg formaldehyde were comprised of 1.5×10^{18} molecules. Thus, the number of formaldehyde molecules dispersed in the 100 cc volume were 1.5×10^{18} or 1.5×10^{16}/cc. Further volume relationships reduced the number

of molecules accordingly, i.e., $1.5 \times 10^{13}/mm^3$ and $1.5 \times 10^4/\mu^3$. Measurement showed that the suspended spore strip occupied 16.6 mm^3 of space. Assuming that the spores were evenly distributed within this volume, then 2.5×10^{14} molecules of monomeric formaldehyde were required to inactivate each spore strip. Further calculations showed that 2.5×10^9 molecules of formaldehyde were required to inactivate each individual spore area (the volume each spore occupied within the filter paper strip). Finally, if an individual bacterial spore occupied 6 μ^3, then 9×10^4 molecules of formaldehyde (4.5×10^{-12} μg) were required to inactivate the spore.

The important aspect of these analytical calculations, which are based on experimental data, is that the findings from studies on spore inactivation by paraformaldehyde at ambient temperature, where the time required for inactivation was considerably prolonged and rate of gaseous evolution considerably different, resulted in comparable findings, i.e., approximately 6×10^4 molecules of monomeric formaldehyde (3×10^{-12} μg) were required to inactivate the bacterial spore. In addition, studies wherein paraformaldehyde-RTV discs were subjected to elevated temperature prior to biologic evaluation of residual sporicidal activity supported observations on the molecular relationship of formaldehyde

gas to spore inactivation. In these studies, discs containing 3.1 mg paraformaldehyde were exposed to 125C for 1 hour during which time approximately 95% (2.95 mg) of added paraformaldehyde had vaporized leaving a residue of approximately 150 μg. These discs were then used for biologic evaluation by placement into 100 cc sealed vessels containing biologic indicators and subjected to 125C for specified time periods. Results showed that a 1-hour exposure period at 125C was sufficient to effect sterility. During this time period approximately 60% or 90 μg of the residual paraformaldehyde had vaporized. Thus, approximately 1×10^5 molecules of monomeric formaldehyde (5×10^{-12} μg) were required to inactivate each bacterial spore.

Additional supportive evidence was provided upon analysis of inactivation data when using melamine formaldehyde at 125C. With the use of 2 mg melamine formaldehyde in a 100 cc volume, 1×10^5 bacterial spores were inactivated in 3 hours. Based on studies of gaseous evolution at 125C, 9.5% of the available formaldehyde had been released during this time period. Because only 53% of the melamine formaldehyde molecule is comprised of formaldehyde residues, calculations showed that approximately 100 μg were released into the 100 cc volume. Therefore,

the number of formaldehyde molecules dispersed per cc of volume was 2×10^{16}. Using this information and the previous calculations on spore volume, the number of monomeric formaldehyde molecules required to inactivate an individual bacterial spore was 1.2×10^{5} (6×10^{-12} μg formaldehyde).

Thus, the maximal number of monomeric formaldehyde molecules required to inactivate a bacterial spore when using paraformaldehyde at ambient temperature or 60C; when using residual paraformaldehyde from heat-treated RTV-sterilant discs by exposure to 125C; or when using melamine formaldehyde at 125C were similar; i.e., 6×10^{4}, 9×10^{4}, 1×10^{5}, and 1.2×10^{5}, respectively. Comparative weight relationships were 3×10^{-12} μg, 4.5×10^{-12} μg, 5×10^{-12} μg, and 6×10^{-12} μg formaldehyde per bacterial spore, respectively. These are conservative values and it is fully recognized that the minimum molecular quantities of monomeric formaldehyde gas required to inactivate a bacterial spore may be several orders of magnitude lower since certain assumptions were made regarding spore distribution and size in the calculations. Since the mechanism of spore inactivation by gaseous formaldehyde is unknown, a priori this information appeared extremely significant. Because 6×10^{4} to 1×10^{5}

molecules of formaldehyde gas were required to inactivate a bacterial spore, it can be hypothesized that for inactivation to occur a large number of intracellular sites have to be attacked by formaldehyde. The obvious intracellular target molecules appear to be the enzymes, of which there are numerous specific copies found intracellularly. Formaldehyde reacts, in general, with proteins by hardening them and decreasing their water-sensitivity (29). Specifically, these tanning effects are due mainly to the cross-linkage of protein chains by methylene bonds containing reactive groups (2). The various protein residues involved in reactions with formaldehyde includes primary amino and amido groups, guanidyl groups, secondary amide groups, indole groups, mercaptan radicals, imidazole groups, and phenolic nuclei (30). Formaldehyde also reacts with nucleic acids and the contribution of these reactions to spore inactivation cannot be discounted. It has been demonstrated that the inactivation of viruses by formaldehyde can result from an aldehyde-nucleic acid reaction (7). Neely (13), in studies on the action of formaldehyde on microorganisms, stated that inactivation may be due to unbalanced growth with the subsequent formation of 1, 3-thiazane-4-cardoxylic acid. He indicated that both nuclear and cytoplasmic syntheses were impaired by this dual action of formaldehyde.

The kinetic studies on the loss of formaldehyde from pure chemicals and from potting compound-sterilant discs exposed to temperatures ranging from ambient to 125C suggested that internal sublimation of sterilants with outward diffusion of evolved formaldehyde gas had taken place. Data indicated that the comparative rates of gaseous evolution from the pure chemicals and from the potting compound-sterilant mixtures were very similar. In addition, the complete vaporization of paraformaldehyde from the paraformaldehyde-RTV discs was certainly indicative of internal evolution, outward diffusion, and ultimate release of formaldehyde gas. Although evolution of gas from paraformaldehyde occurred at a faster rate, eventually going to complete vaporization, as compared to melamine formaldehyde, sufficient sterilizing gas was released from the latter synthetic resin to accomplish the required task of sterilization. The release of monomeric formaldehyde from melamine formaldehyde was limited and even at 125C for 168 hours only 25% of the available formaldehyde was released as a vapor. The remaining formaldehyde residue was apparently bound in such a manner as to preclude release except at pyrolytic temperatures. During the very process of internal production of the sterilant gas, it is anticipated that embedded contaminating microorganisms were subjected to <u>in situ</u> inactivation with the ultimate

sterilization of the entire component. Nevertheless, although experimental data indirectly suggested that internal sterilization of potting compounds was entirely feasible with the use of formaldehyde-liberating additives, what was required was experimental evidence for the verification of internal sterility of potting compound. In addition, the relationship between the concentration of additive and the time required for internal sterility needed to be established at the various experimental temperatures.

After employing several laboratory procedures in an attempt to verify internal sterility, the embedding method previously described was selected as the most reproducible, reliable, and efficient. As reported data indicated, internal sterility was achieved at temperatures ranging from 45 to 90C with paraformaldehyde and from 60 to 90C with melamine formaldehyde. Of the parameters studied, temperature appeared as the most significant factor in effecting sterility. The test procedure used, wherein the bacterial spores are sealed in a 0.5 mil plastic envelope, presented an increased challenge to the sterilization process by imposing a barrier to the passage of sterilant gas. In addition, the concentration of 1×10^5 bacterial spores within a volume of approximately 17 mm^3 would not be expected in potting compounds used in spacecraft electronic components.

Nevertheless, even under these stringent challenge conditions, sterility was achieved at 90C within 30 minutes and 2 hours, respectively, when using 1% paraformaldehyde or 1.9% melamine formaldehyde. According to data on gaseous evolution, less than 10% of the available formaldehyde was released by the additives during the period required for internal sterilization. Therefore, it is fully anticipated that concentrations of additive considerably lower than 1% could be used with extended sterilization periods.

The interesting chemical DMSO possesses a duality of properties that prompted us to consider this material as a potential enhancement factor in the sterilization of potting compound. These properties include the capacity of DMSO to act as a penetrant carrier (15) and its bactericidal (3) and virucidal (5) activities. Thus, the combined action of DMSO and sterilant gas could theoretically result in an enhancement of sporicidal activity. The mechanism of action of DMSO could be as a penetrant carrier for monomeric formaldehyde (increased penetrability of potting compound or bacterial spore wall) or as an additive biocidal agent. Studies to date, although preliminary, indicated that the combined use of DMSO and formaldehyde gas was associated with greater sporicidal activity than when either agent was used alone. Additional studies are contem-

plated to obtain more definitive information on the mechanism of action involved.

The concept of a "self-sterilizing" potting compound has been demonstrated by the studies presented in this report. Procedures were developed that could ensure the internal sterility of electronic spacecraft components. The applicability to the NASA spacecraft sterilization program could result in a further reduction of the total microbial burden of the spacecraft, thereby allowing a shorter and less thermally destructive heat sterilization cycle.

ACKNOWLEDGEMENT

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FUTURE WORK

During the course of this contract (NASw-1764) a new contract (NAS8-24513) was entered into between the Marshall Space Flight Center (MSFC) and Becton, Dickinson and Company. The current contract research systematically extends the studies reported herein.

The MSFC contract will include an investigation of the basic parameters of paraformaldehyde sterilization, i.e., the influence of environmental and cellular parameters on the inactivation of bacterial spores by formaldehyde gas. Specifically the role of relative humidity and cellular water content will be studied in relation to gaseous inactivation. Simulant spacecraft component pieces will be examined physically and functionally by MSFC after exposure to the required elevated temperature for the release of the sterilizing gas and upon demonstration by the Contractor that the sterilization of subject component pieces had taken place. Additional organic resins and polymers selected jointly by MSFC and the Contractor, will be investigated. Thus, the specific goal of this program is to develop a useful and reliable method for sterilization of

potting compounds, mated and exposed spacecraft surfaces, occluded areas and electronic systems at temperatures ranging from 60 to 125C.

TABLE I

BIOCIDAL PROPERTIES OF MELAMINE FORMALDEHYDE

Melamine formaldehyde[a] (mg)	Microbial growth[b] after exposure at				
	22C	35C	45C	60C	125C
2	+	+	+	+	-
4	+	+	+	+	-
6	+	+	±	±	-

(a) Per 100 cc air; 24-hour exposure.

(b) Biologic indicators with 1 x 10^5 B. stearothermophilus spores/strip suspended in sealed glass vessels.

TABLE II

BIOCIDAL PROPERTIES OF UREA FORMALDEHYDE AT 60C

Urea formaldehyde (a) (mg)	Test bacterium (b)	Microbial growth after indicated exposure (hrs)					
		1	2	3	4	5	6
2.5	B. globigii	+	±	-	-	-	-
	B. stearothermophilus			+	+	+	+
5.0	B. globigii	+	-	-	-	-	-
	B. stearothermophilus	+	+	+	-	-	-

(a) Per 100 cc air; used as 5-10% aqueous solution.

(b) Biologic indicators with 1×10^5 bacterial spores/strip suspended in sealed glass vessels.

TABLE III

STERILIZING ACTIVITY OF 1% AQUEOUS SOLUTION

OF UREA FORMALDEHYDE

Time[a] (hrs)	Microbial growth[b] after exposure at		
	22C	45C	60C
0	+	+	+
1	+	-	-
2	+	-	-
3	+	-	-

(a) Exposure time at indicated temperature after spray application of 1% urea formaldehyde.

(b) B. globigii on metal strips.

TABLE IV

BIOCIDAL PROPERTIES OF PARAFORMALDEHYDE[a]

Time (hrs)	Microbial growth[b] after exposure at			
	22C	35C	60C	125C
0.5	+	+	+	+
1	+	+	+	-
3	+	+	+	-
5	+	+	-	-
7	+	+	-	-
14	+	-	-	-
72	-	-	-	-

(a) 1 mg/100 cc air.

(b) Biologic indicators with 1 x 10^5 B. stearothermophilus spores/strip suspended in sealed glass vessels.

TABLE V

BIOCIDAL ACTIVITY OF PARAFORMALDEHYDE[a] AT 35C

Time[b] (hrs)	Microbial growth[c] at indicated incubation time (hrs)		
	24	48	96
4-7	+		
9-10	-	+	
12	-	-	+
14-24	-	-	-

(a) 2 mg/100 cc air.

(b) Exposure time in sealed glass vessel.

(c) Biologic indicators with 1×10^5 B. stearothermophilus spores/strip.

TABLE VI

INACTIVATION OF BACILLUS STEAROTHERMOPHILUS AND BACILLUS GLOBIGII

BY MELAMINE FORMALDEHYDE OR PARAFORMALDEHYDE AT 125C

Time[b] (hrs)	Microbial growth after indicated exposure			
	Melamine formaldehyde[a]		Paraformaldehyde[a]	
	B. stearothermophilus	B. globigii	B. stearothermophilus	B. globigii
0.5	+	+	+	+
1.0	+	+	-	±
1.5	+	+	-	-
2.0	+	±	-	-
2.5	+	±	-	-
3.0	-	±	-	-

(a) 2 mg/100 cc air.

(b) Exposure time in sealed glass vessels.

TABLE VII

MICROBIAL INHIBITION BY POTTING COMPOUND[a]-STERILANT MIXTURES

Bacterium	Inhibition zones[b] (mm) with indicated additive					
	Paraformaldehyde		Melamine formaldehyde		Urea formaldehyde	
	1%	5%	1%	5%	1%	5%
B. globigii	25	34	10	16	10	16
S. marcescens	15	26	10	12	10	10
K. pneumoniae	21	29	10	10	10	15
S. aureus	32	44	16	20	15	20
E. coli	17	26	10	17	10	10

(a) RTV-3140.

(b) Modification of classical antibiotic sensitivity procedure; discs 10 mm in diameter.

TABLE VIII

LOSS OF FORMALDEHYDE FROM PARAFORMALDEHYDE AND MELAMINE FORMALDEHYDE AT ELEVATED TEMPERATURES

Exposure time (hrs)	Percent loss at indicated exposure temperature							
	45C		60C		90C		125C	
	PF	MF	PF	MF	PF	MF	PF	MF
1			1.0	2.0	19.5	4.0	95.1	5.8
2			1.7	2.4	36.0	5.6	98.1	9.0
3			4.3	2.6	41.3	5.4	98.6	9.2
4			5.4	3.0	45.6	5.6	98.8	10.0
5			7.5	2.6	47.3	8.4	99.0	9.8
6			11.3	2.8	52.3	9.0	99.5	10.6
12			12.7	3.6	56.3	7.2	100.0	19.2
24	5.7		24.0	3.0	82.0	9.8		19.8
48	14.1	4.4	38.9	3.4	95.5	9.6		20.8
72	23.5	4.4	49.5	10.0	98.1	11.0		21.2
168	54.2	6.8	73.4	10.8	99.8	14.4		24.2

TABLE IX

INACTIVATION OF EMBEDDED BACTERIAL SPORES AT 45C BY FORMALDEHYDE GAS

Exposure time (hrs)	Microbial growth[1] after exposure to		
	RTV	RTV-MF[2]	RTV-PF[3]
0	+	+	+
6			+
12			±
24	+	+	-
48	+	+	-
72	+	+	-
96	+	±	-

(1) Biologic indicators with 1 x 10^5 bacterial spores/strip.

(2) Melamine formaldehyde (9.5%).

(3) Paraformaldehyde (5%).

TABLE X

INACTIVATION OF EMBEDDED BACTERIAL SPORES AT 45C BY FORMALDEHYDE GAS

Exposure time (hrs)	Microbial growth[1] after exposure to		
	RTV	RTV-MF[2]	RTV-PF[3]
0	+	+	+
6			+
12			+
18			±
24	+	+	-
48	+	+	-
72	+	+	-
96	+	+	-

(1) Biologic indicators with 1 x 10^5 bacterial spores/strip.

(2) Melamine formaldehyde (1.9%).

(3) Paraformaldehyde (1%).

TABLE XI

INACTIVATION OF EMBEDDED BACTERIAL SPORES AT 60C BY FORMALDEHYDE GAS

Exposure time (hrs)	Microbial growth[1] after exposure to		
	RTV	RTV-MF[2]	RTV-PF[3]
0	+	+	+
1			±
2			-
3			-
5			-
7			-
12		±	
18		+	
24	+	±	
36	+	-	
48	+	-	
72	+	-	
96	+	-	

(1) Biologic indicators with 1×10^5 bacterial spores/strip
(2) Melamine formaldehyde (9.5%).
(3) Paraformaldehyde (5%).

TABLE XII

INACTIVATION OF EMBEDDED BACTERIAL SPORES AT 60C BY FORMALDEHYDE GAS

Exposure time (hrs)	Microbial growth[1] after exposure to		
	RTV	RTV-MF[2]	RTV-PF[3]
0	+	+	+
3			+
5			+
6			-
7			-
12			-
24	+	+	-
48	+	+	-
72	+	+	-
96	+	+	-

(1) Biologic indicators with 1×10^5 bacterial spores/strip.
(2) Melamine formaldehyde (1.9%).
(3) Paraformaldehyde (1%).

TABLE XIII

INACTIVATION OF EMBEDDED BACTERIAL SPORES AT 90C BY FORMALDEHYDE GAS

Exposure time	Microbial growth[1] after exposure to		
	RTV	RTV-MF[2]	RTV-PF[3]
0	+	+	+
15 min		+	-
30 "		+	-
45 "			-
1 hr	+	-	-
2 "	+		-
3 "	+	-	-
5 "		-	
6 "		-	
12 "	+		
18 "	±		
24 "	±	-	

(1) Biologic indicators with 1 x 10^5 bacterial spores/strip.
(2) Melamine formaldehyde (9.5%).
(3) Paraformaldehyde (5%).

TABLE XIV

INACTIVATION OF EMBEDDED BACTERIAL SPORES AT 90C BY FORMALDEHYDE GAS

Exposure time	Microbial growth[1] after exposure to		
	RTV	RTV-MF[2]	RTV-PF[3]
0	+	+	+
15 min			±
30 "			-
45 "			-
1 hr	+		-
2 "	+	-	-
3 "	+		
4 "		-	
6 "	+	-	
18 "	+		
24 "	+	-	

(1) Biologic indicators with 1×10^5 bacterial spores/strip.
(2) Melamine formaldehyde (1.9%).
(3) Paraformaldehyde (1%).

TABLE XV

SUMMARY ON INACTIVATION OF EMBEDDED BACTERIAL SPORES

BY FORMALDEHYDE LIBERATING ADDITIVES

Exposure temperature	Time required to achieve internal sterility[1]			
	Melamine formaldehyde		Paraformaldehyde	
	9.5%	1.9%	5%	1%
45C	~96 hrs	>96 hrs	24 hrs	24 hrs
60C	36 hrs	~72 hrs	2 hrs	6 hrs
90C	1 hr	2 hrs	15 min	30 min

(1) 1×10^5 Bacillus stearothermophilus spores used as challenge

TABLE XVI

COMBINED SPORICIDAL ACTIVITY OF PARAFORMALDEHYDE AND DIMETHYLSULFOXIDE (DMSO)

Test condition	Microbial growth[b] after exposure (min) at indicated temperature							
	90C					60C		
	15	30	60	90	120	180	240	300
Paraformaldehyde[a]	+	+	+	±	-	+	+	-
DMSO	+	+	±	±	-	+	+	+
Paraformaldehyde[a] + DMSO	+	±	-	-	-	-	-	-
None	+	+	+	+	+	+	+	+

(a) 1 mg/100 cc air.

(b) Biologic indicators with 1 x 10^5 bacterial spores/strip suspended in sealed glass vessels.

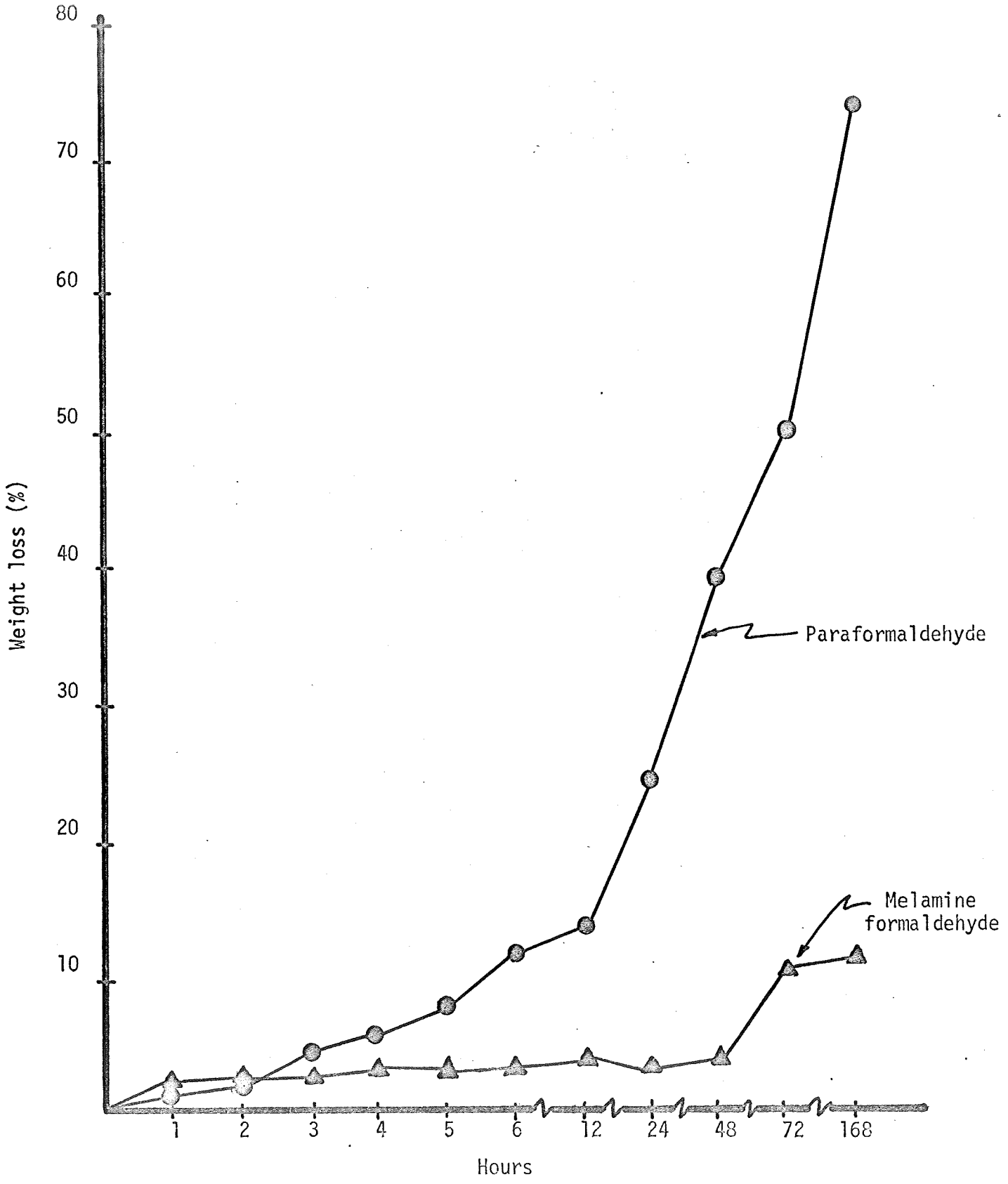


FIGURE 1. LOSS OF FORMALDEHYDE FROM PARAFORMALDEHYDE AND MELAMINE FORMALDEHYDE AT 60C

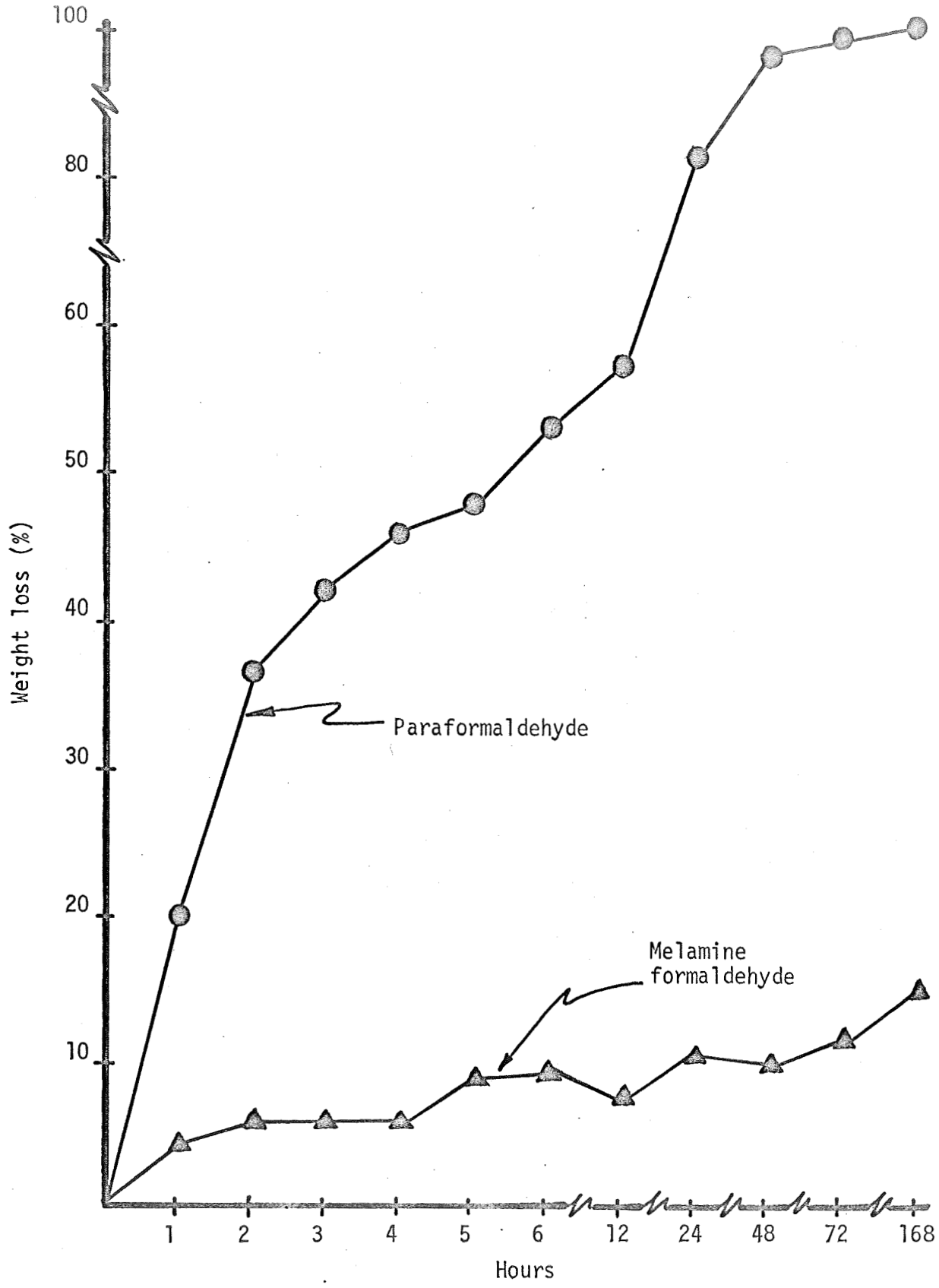


FIGURE 2. LOSS OF FORMALDEHYDE FROM PARAFORMALDEHYDE AND MELAMINE FORMALDEHYDE AT 90C

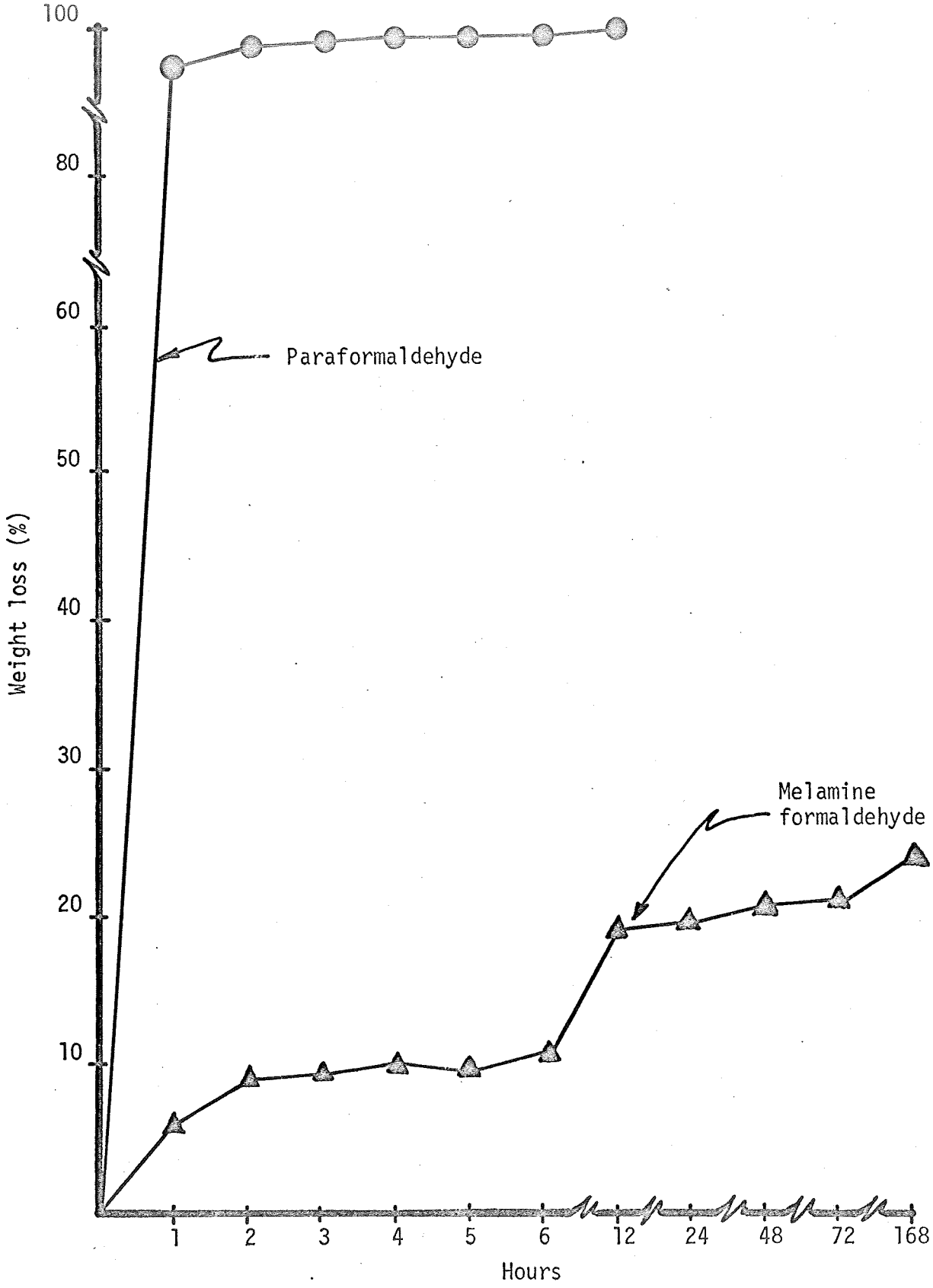


FIGURE 3. LOSS OF FORMALDEHYDE FROM PARAFORMALDEHYDE AND MELAMINE FORMALDEHYDE AT 125C

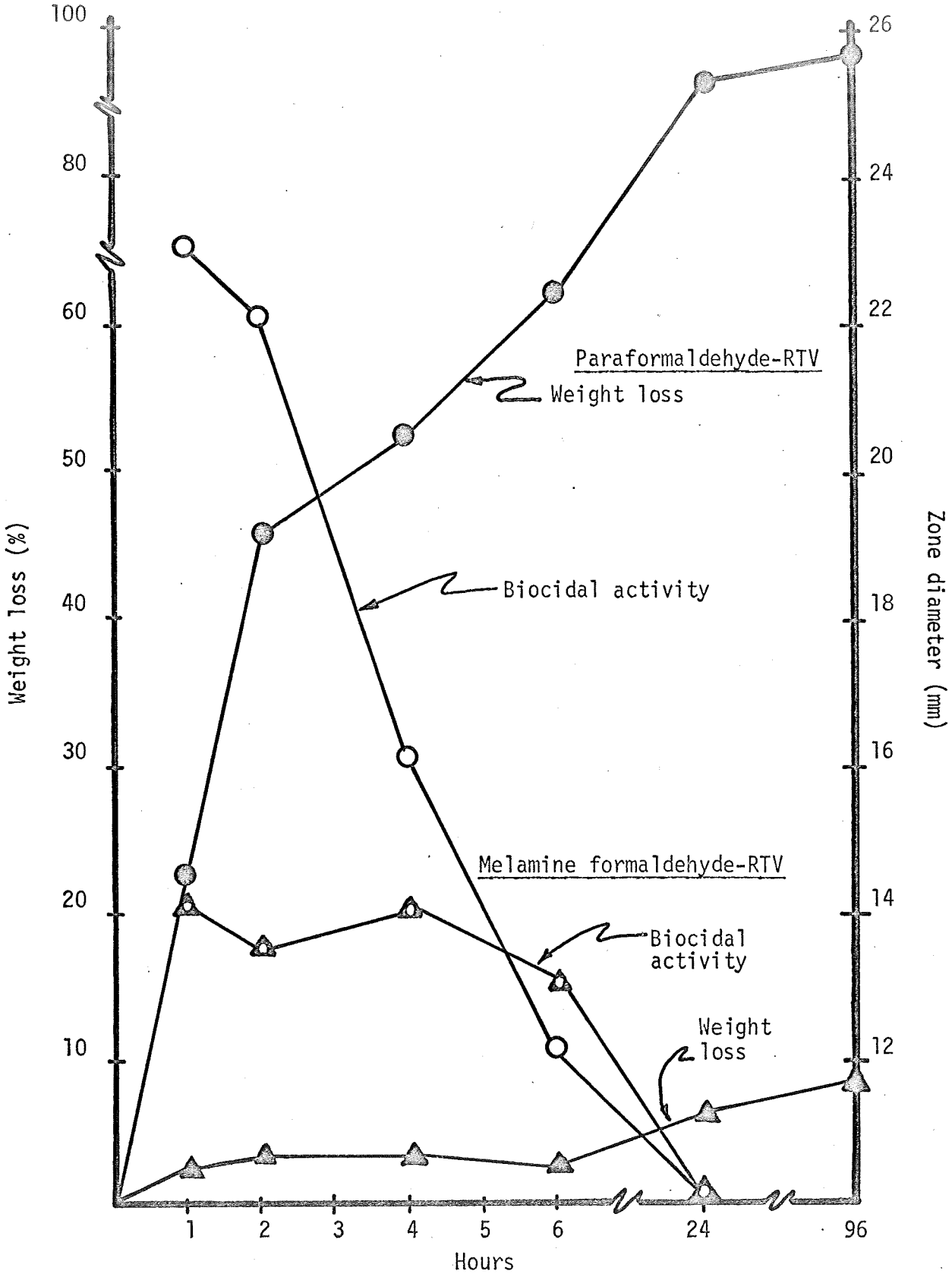


FIGURE 4. LOSS OF FORMALDEHYDE AND BIOCIDAL ACTIVITY FROM PARAFORMALDEHYDE-RTV AND MELAMINE FORMALDEHYDE-RTV AT 90C

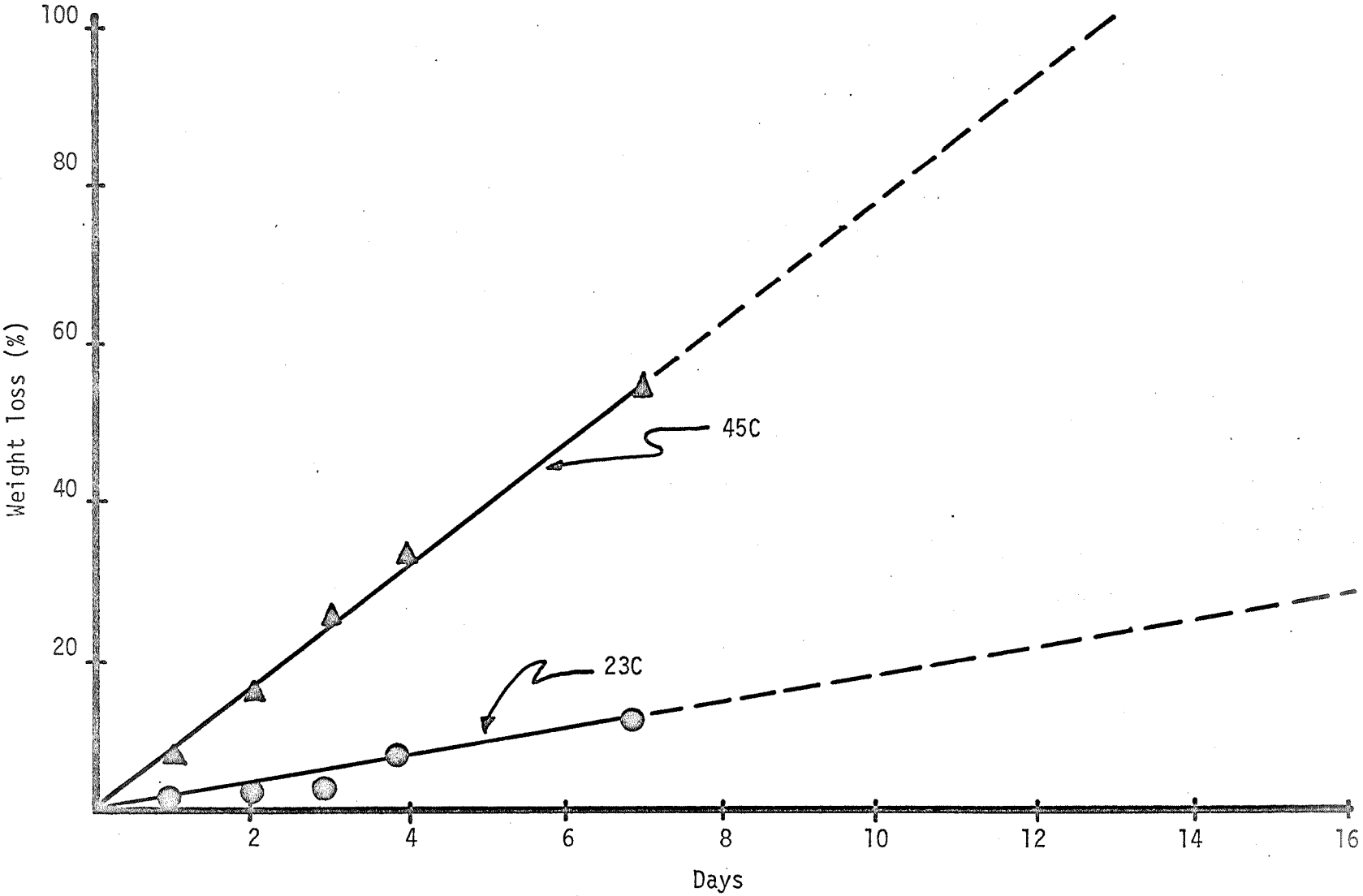


FIGURE 5. LOSS OF FORMALDEHYDE GAS FROM PARAFORMALDEHYDE AT AMBIENT TEMPERATURE AND 45C

Figure 6. Characteristic Inhibition of Microbial Growth by RTV-Sterilant Discs

No.	Test Organism	Additive
1	Staphylococcus aureus	None
2	Staphylococcus aureus	Melamine formaldehyde (19%)
3	Staphylococcus aureus	Paraformaldehyde (10%)
4	Escherichia coli	None
5	Escherichia coli	Melamine formaldehyde (19%)
6	Escherichia coli	Paraformaldehyde (10%)

REFERENCES

1. Aldridge, C., NASA Spacecraft Sterilization Technology Seminar, Southwestern Radiological Health Laboratory, Las Vegas (1969).

2. Anson, M. L. and Edsall, J. T., "Advances in Protein Chemistry", II, p. 278-336, New York Academic Press (1945).

3. Basch, H. and Gadebusch, H. H., Appl. Microbiol., 16, No. 12, 1953-1954 (1968).

4. Butlerov, A., Ann., 111, 245, 247-248 (1859).

5. Chan, J. C. and Gadebusch, H. H., Appl. Microbiol., 16, No. 10, 1625-1626 (1968).

6. Dunklin, E. W. and Lester, W. T., Soap, N. Y., 27, 127 (1951).

7. Fraenkel-Conrat, H., Biochem. Biophys. Acta., 15, 307-309 (1954).

8. Hoffman, R. K., Proc. Chem. Spec. Manuf. Assoc., N. Y. (1951).

9. Hoffman, R. K., Kaye, S., and Feazel, C. E., Official Digest, Fed. of Paint and Varnish Prod. (1959).

10. Hoffman, R. K., Yeager, S. B., and Kaye, S., Soap and Chem. Spec., 31, No. 8, 135 (1955).

11. Kaitz, C., U. S. Patent 2,993,832, Poultry and Egg Fumigation Process, July 25, 1961.

12. Morris, E. J. and Darlow, H. M., J. Appl. Bacteriol., 1, 64-72 (1959).

13. Neely, W. B., J. Bacteriol., 86, 445-448 (1963).

14. Nordgren, G., Acta. Path. Microbiol. Scand. (suppl.), 40, 21-34 (1939).

15. Pottz, G. E., Rampey, J. A., and Benjamin, F., Ann. N. Y. Acad. Sci., 141, 261-272 (1967).

16. Skelly, J. K., Chem. and Ind., 50-56 (Jan., 1965).

17. Solomon, G. L., Proc. Amer. Assoc. Textile Chemists and Colorists, Amer. Dyestuff Reporter, 29-34 (Dec., 1968).

18. Taylor, L. A., Barbeito, M. S., and Gremillion, G. G. Appl. Microbiol., 17, No. 4, 614-618 (1969).

19. Tollen, S. B. and Mayer, F., Ber., 21, 1566, 2026, and 3503 (1888).

20. Vineland Poultry Laboratories, "Fumigation with Formaldegen", Bulletin R5-767030, Vineland, New Jersey.

21. Walker, J. F., "Formaldehyde", III, p. 53, New York, Reinhold Pub. Corp. (1964).

22. Ibid., p. 145.

23. Ibid., p. 147.

24. Ibid., p. 149.

25. Ibid., p. 304.

26. Ibid., p. 305.

27. Ibid., p. 336.

28. Ibid., pp. 337-378.

29. Ibid., p. 399.

30. Ibid., p. 400.

APPENDIX

TABLE I

PROPERTIES OF PARAFORMALDEHYDE*

Assay, as formaldehyde, wt %, min	95.0
Ash, wt %, max	0.01
Iron, ppm by wt, max	2.0
Acidity, as formic, wt%, max	0.03
Water, wt %, max	5.0
Appearance	White solid
Odor	Pungent
Flash point, Tag open cup, F approx.	200
Ignition temperature, F approx.	575
Methanol content	None
Explosive limits for formaldehyde gas: Per cent by volume in air,	
Lower limit	7.0
Upper limit	73.0
Molecular weight, approx.	600

Solubility of paraformaldehyde is a function of pH and temperature; most soluble at pH extremes and reflux temperature.

* "Paraformaldehyde", Celanese Chemical Company, Division of Celanese Corporation, New York, N. Y. (1967).

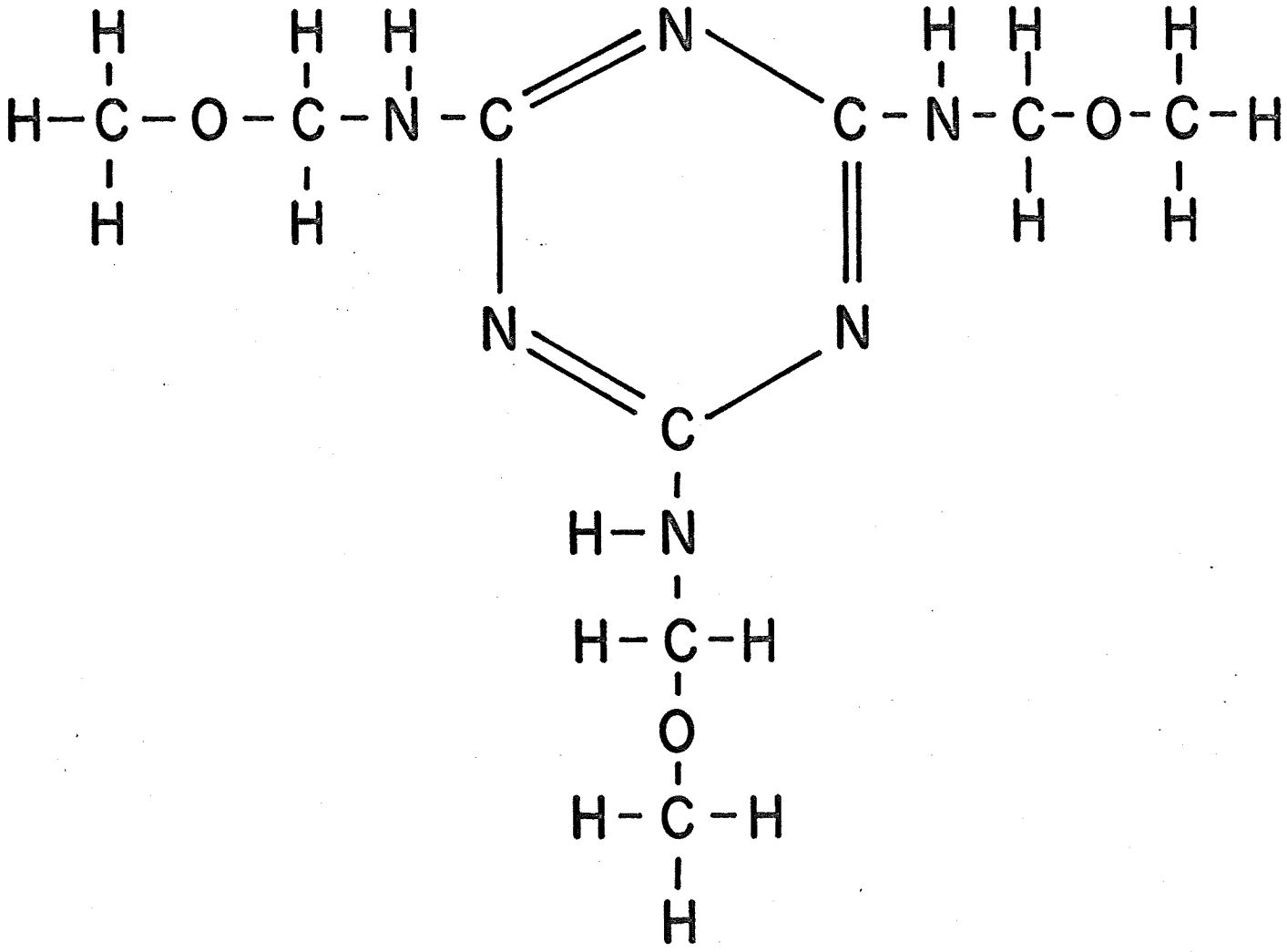
APPENDIX

TABLE II

WEIGHT LOSS OF RTV DURING CURING PROCESS AT AMBIENT TEMPERATURE

Sample	Weight loss (%) at indicated time (hrs)					
	24	48	72	120	168	336
1	.88	1.08		1.25	1.29	1.33
2	.93	1.14		1.30	1.34	1.38
3	.90	1.12		1.28	1.31	1.36
4	.90	1.09	1.18	1.27	1.32	
5	.89	1.09	1.18	1.27	1.31	
6	.90	1.10	1.19	1.28	1.33	

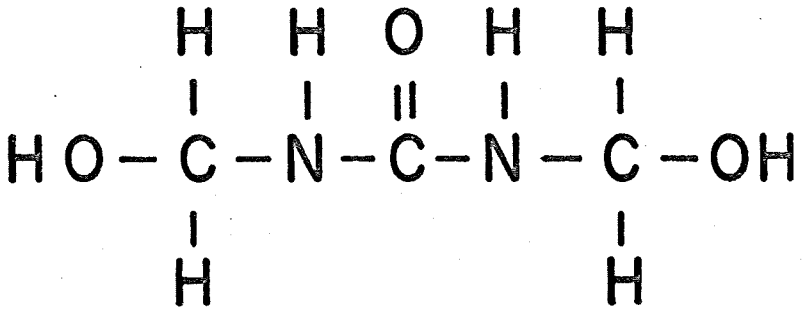
MELAMINE FORMALDEHYDE



APPENDIX

Figure 1: Structure of Melamine Formaldehyde

DIMETHYLOL UREA

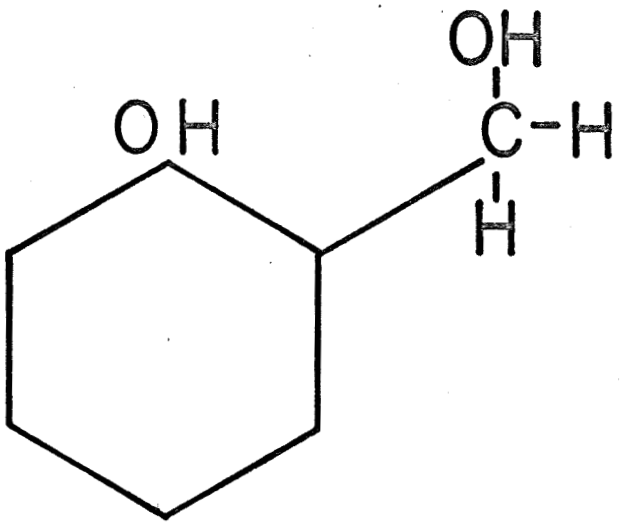


APPENDIX

Figure 2: Structure of Urea Formaldehyde

PHENOL FORMALDEHYDE

ORTHO-METHYLOLPHENOL



METHYLENE PHENOL

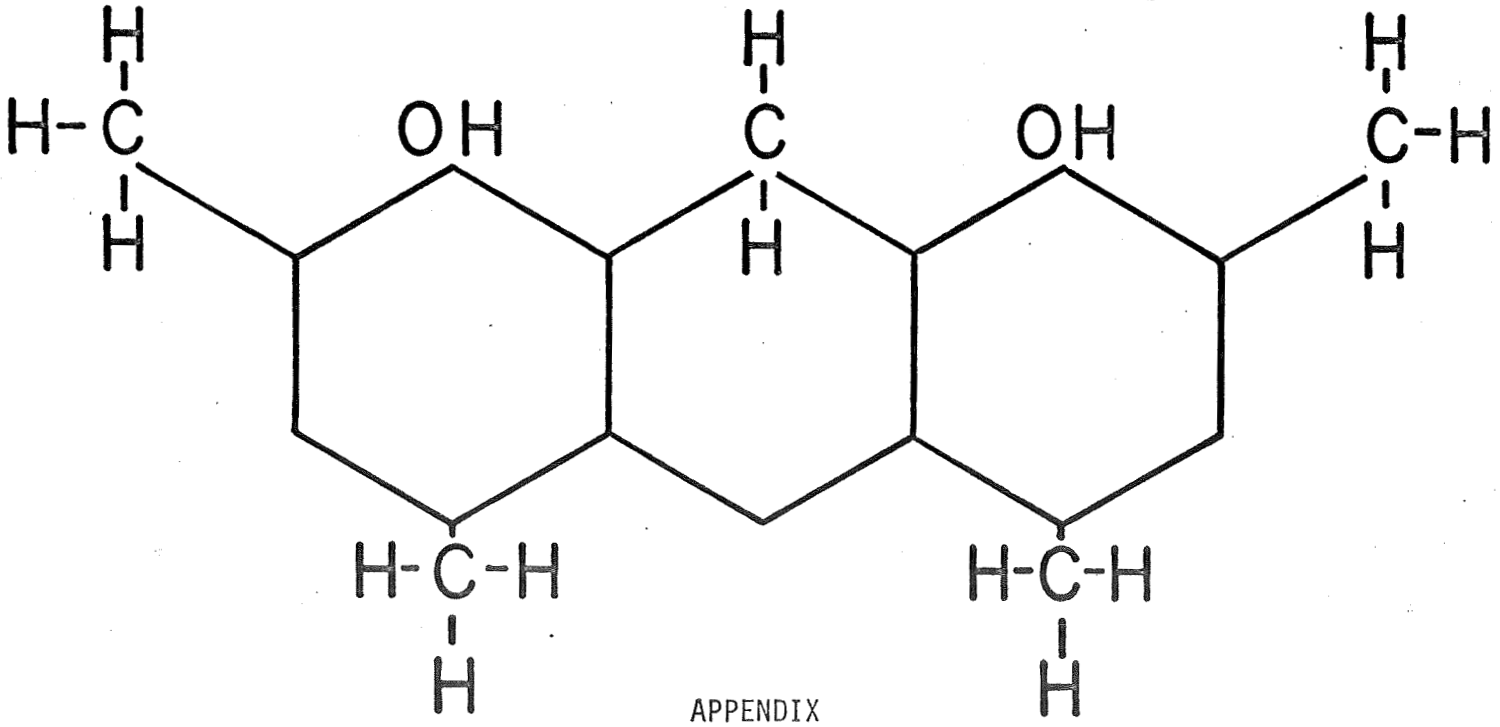


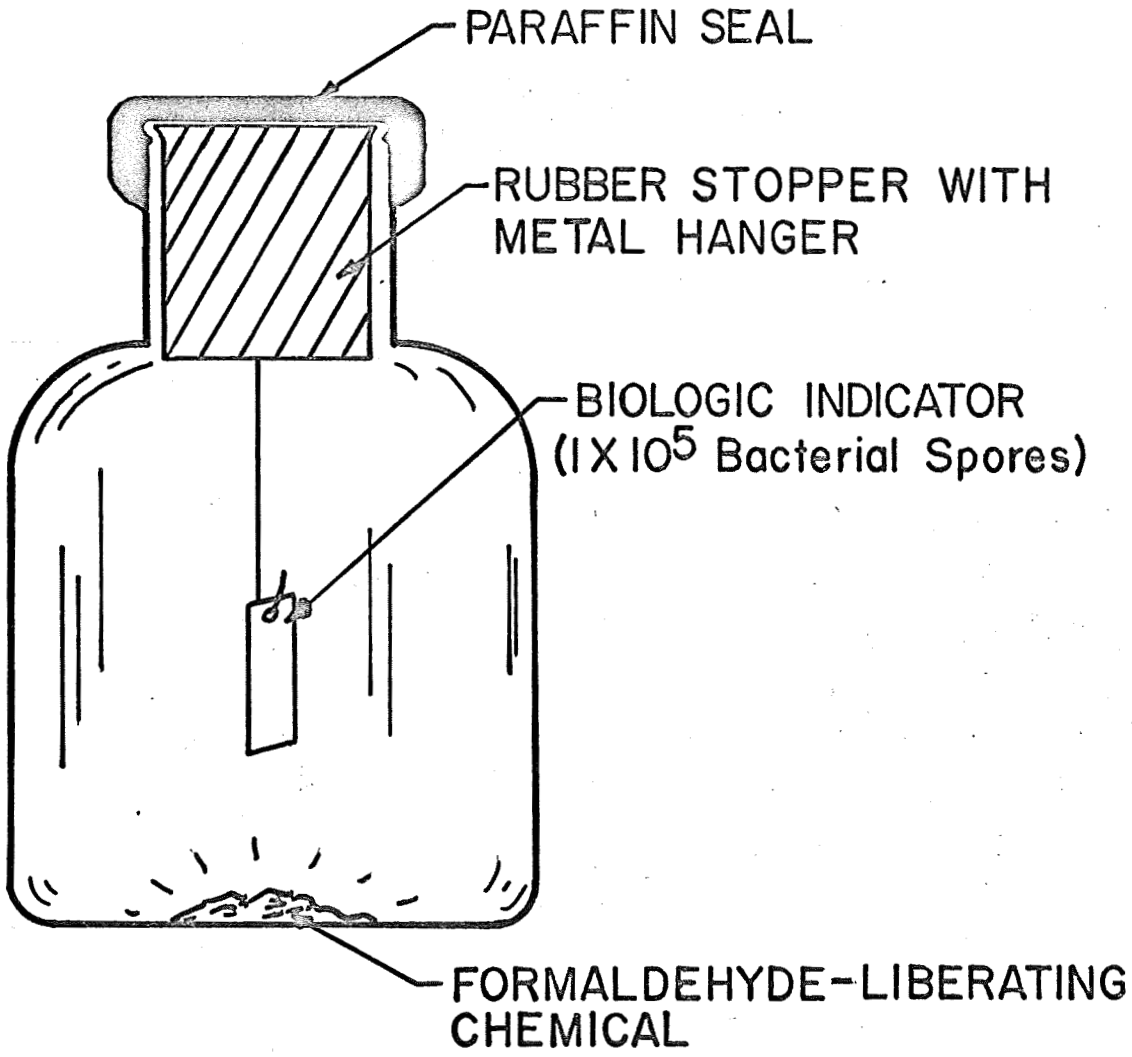
Figure 3: Structure of Phenol Formaldehyde

PARAFORMALDEHYDE

$$HO-(CH_2O)_n-H$$

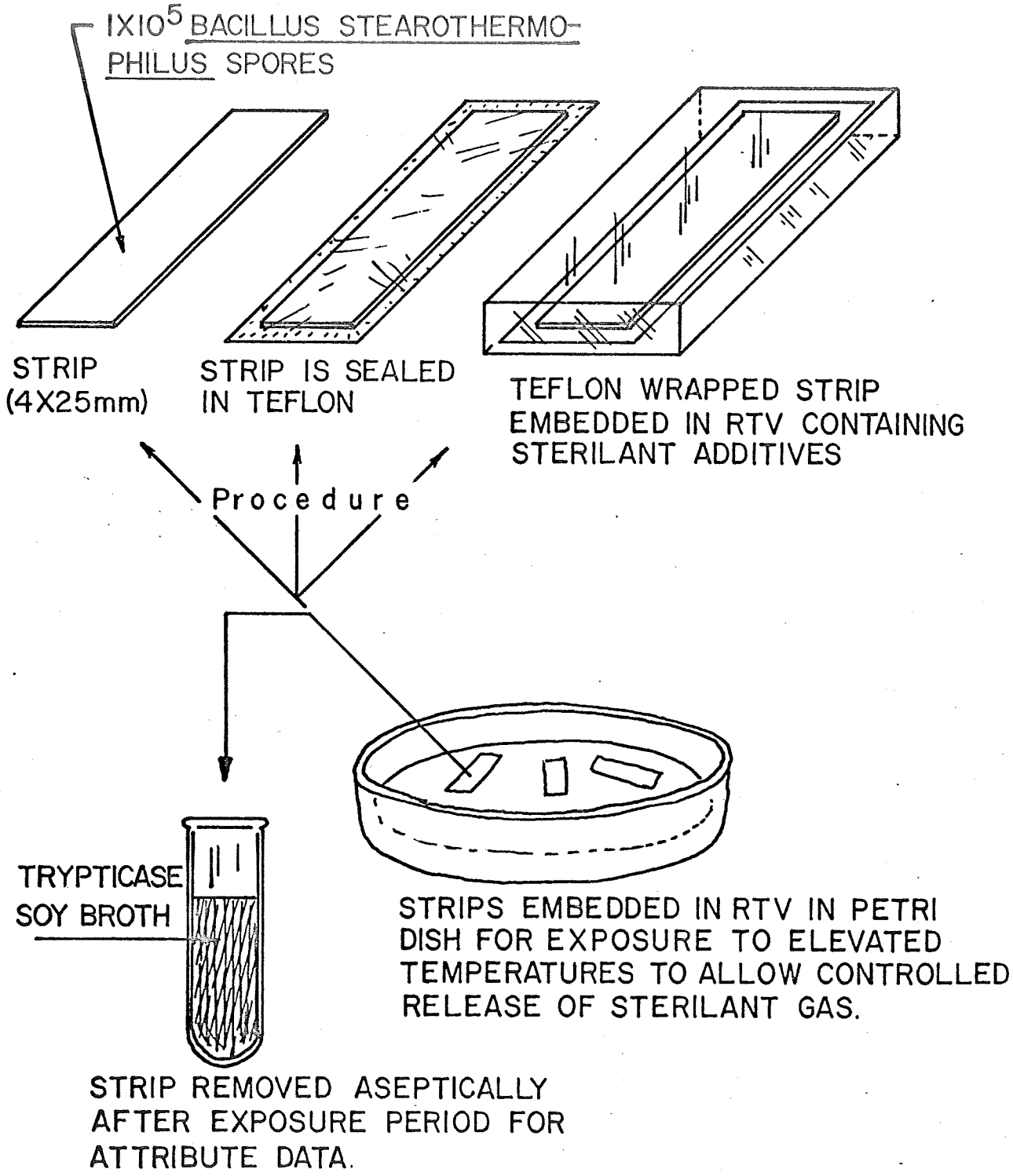
APPENDIX

Figure 4: Structure of Paraformaldehyde



APPENDIX

Figure 5: Test Procedure for Evaluation of Formaldehyde-Liberating Chemicals



APPENDIX

Figure 6: Embedding Procedure for Verification of Internal Sterility of Potting Compound